

ANalysis Of VAriance

Presenter- Dr. SNEH KHATRI

Junior Resident

PGIMS Rohtak

Contents

- Introduction – Various statistical tests
- What is ANOVA?
- One way ANOVA
- Two way ANOVA
- MANOVA (Multivariate ANalysis Of VAriance)
- ANOVA with repeated measures
- Other related tests
- References

Summary Table of Statistical Tests

Level of Measurement	Sample Characteristics					Correlation
	1 Sample	2 Sample		K Sample (i.e., >2)		
		Independent	Dependent	Independent	Dependent	
Categorical or Nominal	χ^2 or binomial	χ^2	Macnarmar's χ^2	χ^2	Cochran's Q	
Rank or Ordinal	χ^2	Mann Whitney U	Wilcoxin Matched Pairs Signed Ranks	Kruskal Wallis H	Friedman's ANOVA	Spearman's rho
Parametric (Interval & Ratio)	z test or t test	t test between groups	t test within groups	1 way ANOVA between groups	1 way ANOVA (within or repeated measure)	Pearson's r
		Factorial (2 way) ANOVA				

What is ANOVA



- ✓ Statistical technique specially designed to test whether the means of more than 2 quantitative populations are equal.
- ✓ Developed by Sir Ronald A. Fisher in 1920's.



EXAMPLE: Study conducted among men of age group 18-25 year in community to assess effect of SES on BMI

Lower SES	Middle SES	Higher SES
18,17,18,19,19	22,25,24,26,24,21	25,26,24,28,29
N1= 5	N2= 6	N3= 5
Mean=18.2	Mean= 23.6	Mean=26.4

ANOVA

One way ANOVA

Two way ANOVA

Three way ANOVA

Effect of SES on BMI

Effect of age & SES on BMI

Effect of age, SES, Diet on BMI

ANOVA with repeated measures - comparing ≥ 3 group means where the participants are same in each group. E.g.

Group of subjects is measured more than twice, generally over time, such as patients weighed at baseline and every month after a weight loss program

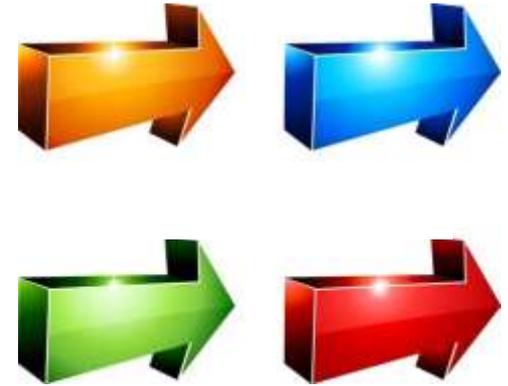


One Way ANOVA

Data required

One way ANOVA or single factor ANOVA:

- Determines means of **≥ 3 independent groups** significantly different from one another.



- Only 1 independent variable (factor/grouping variable) with ≥ 3 levels
- Grouping variable- nominal
- Outcome variable- interval or ratio

Post hoc tests help determine where difference exist

Assumptions

Skewness

Kurtosis

Kolmogorov-Smirnov

Shapiro-Wilk test

Box-and-whiskers plots

Histogram

- 1) **Normality:** The values normally distributed.
- 2) **Homogeneity of variance:** within each group should be equal for all groups.
- 3) **Independence of error:** The error (variation of each value around its own group mean) should be independent for each value.

Steps

1. State null & alternative hypotheses

2. State Alpha

3. Calculate degrees of Freedom

4. State decision rule

5. Calculate test statistic

- Calculate variance between samples

- Calculate variance within the samples

- Calculate F statistic

6. State results & conclusion *post hoc* test

1. State null & alternative hypotheses

$$H_0 = \mu_1 = \mu_2 \dots = \mu_i$$

H0 : all sample means are equal

H_a = not all of the μ_i are equal

At least one sample has different mean

2. State Alpha i.e 0.05

3. Calculate degrees of Freedom

$K-1$ & $n-1$

k = No of Samples,

n = Total No of observations

4. State decision rule

If calculated value of $F >$ table value of F , reject H_0

5. Calculate test statistic

Calculating variance between samples

1. Calculate the **mean** of each sample.
2. Calculate the **Grand average**
3. Take the difference between means of various samples & grand average.
4. Square these deviations & obtain total which will give sum of squares between samples **(SSC)**
5. Divide the total obtained in step 4 by the degrees of freedom to calculate the mean sum of square between samples **(MSC)**.

Calculating Variance within Samples

1. Calculate **mean** value of each sample
2. Take the deviations of the various items in a sample from the mean values of the respective samples.
3. Square these deviations & obtain total which gives the sum of square within the samples **(SSE)**
4. Divide the total obtained in 3rd step by the degrees of freedom to calculate the mean sum of squares within samples **(MSE)**.

The mean sum of squares

Calculation of **MSC**-
Mean sum of Squares
between samples

$$MSC = \frac{SSC}{k - 1}$$

Calculation of **MSE**
Mean Sum Of
Squares within
samples

$$MSE = \frac{SSE}{n - k}$$

k= No of Samples, n= Total No of observations

Calculation of F statistic

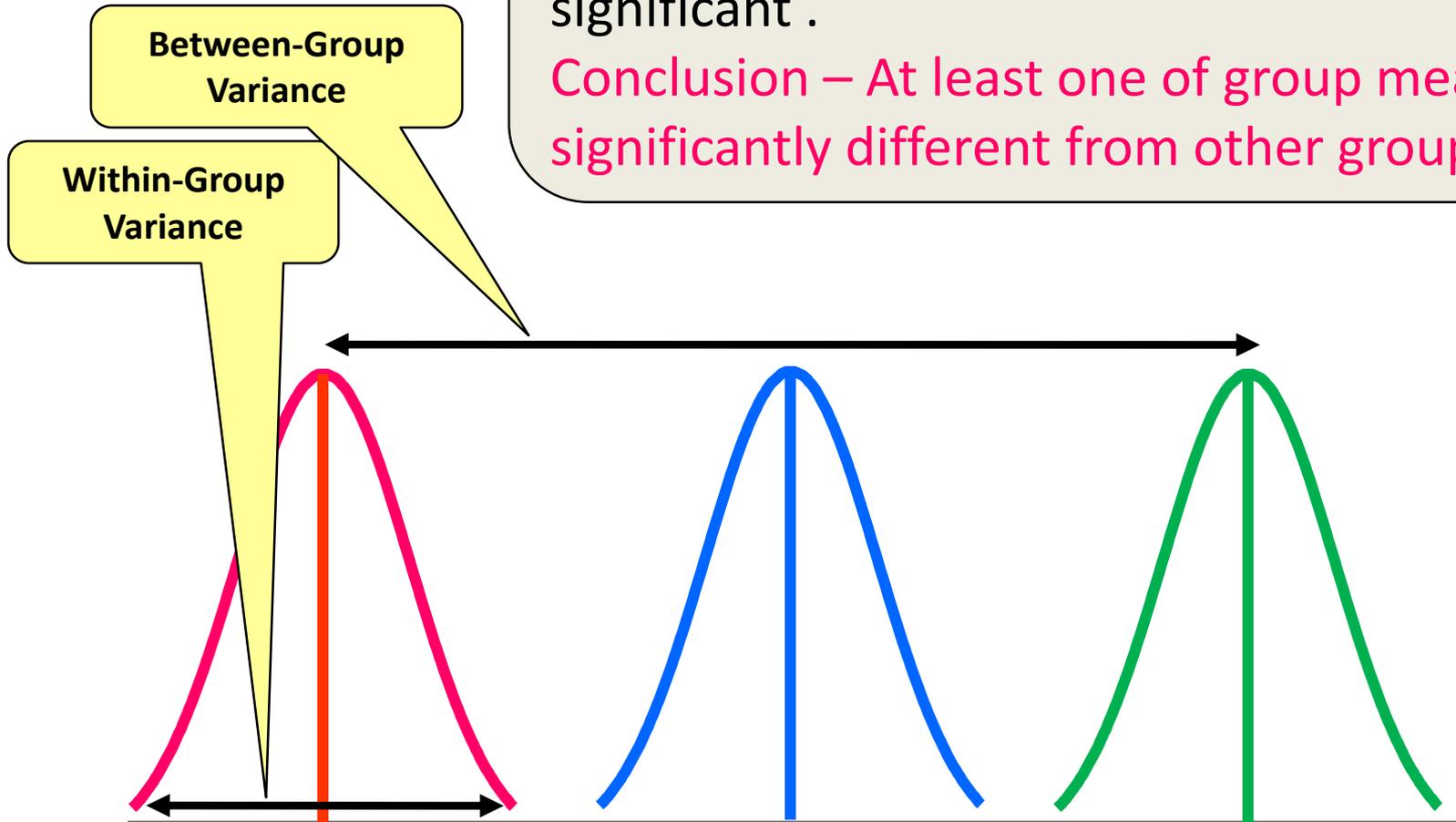
$$F = \frac{\text{Variability between groups}}{\text{Variability within groups}}$$

$$F\text{- statistic} = \frac{MSC}{MSE}$$

Compare the F-statistic value with F(critical) value which is obtained by looking for it in F distribution tables against degrees of freedom. The calculated value of $F >$ table value H_0 is rejected

Between-group variance is large relative to the within-group variance, so F statistic will be larger & $>$ critical value, therefore statistically significant .

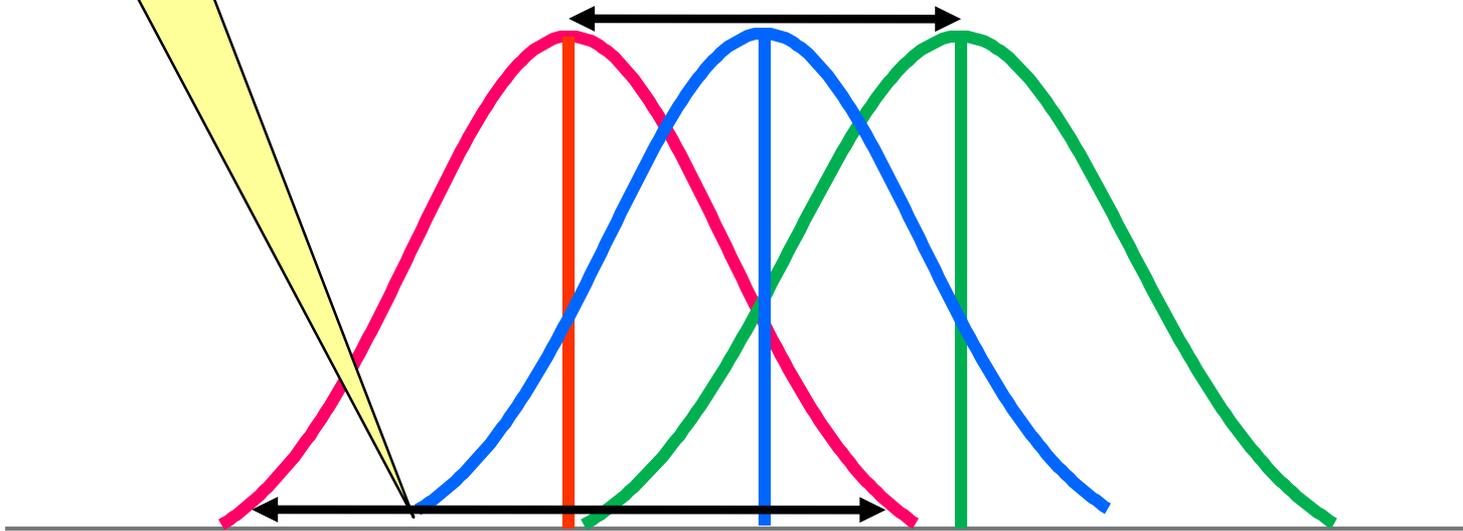
Conclusion – At least one of group means is significantly different from other group means



Within-group variance is larger, and the between-group variance smaller, so F will be smaller (reflecting the likely-hood of no significant differences between these 3 sample means)

Between-Group Variance

Within-Group Variance



Post-hoc Tests

- Used to determine which mean or group of means is/are significantly different from the others (**significant F**)
- Depending upon research design & research question:
 - ✓ **Bonferroni** (more powerful)
Only some pairs of sample means are to be tested
Desired alpha level is divided by no. of comparisons
 - ✓ **Tukey's HSD Procedure**
when all pairs of sample means are to be tested
 - ✓ **Scheffe's Procedure** (when sample sizes are unequal)

One way ANOVA: Table

Source of Variation	SS (Sum of Squares)	Degrees of Freedom	MS (Mean Square)	Variance Ratio of F
Between Samples	SSC			
Within Samples	SSE			
Total	SS(Total)			

The diagram shows a vertical stack of 12 colored squares (4 green, 4 red, 4 blue) on the left, with a long double-headed arrow indicating the total height. This is followed by an equals sign. To the right of the equals sign are three separate vertical stacks of 4 squares each (green, red, blue), with a horizontal double-headed arrow below them indicating the width of the groups. This is followed by a plus sign. To the right of the plus sign are three separate vertical stacks of 4 squares each (green, red, blue), with a vertical double-headed arrow next to each stack indicating the height of each group. Below the diagram is the equation: Total Sum of Squares = Sum of Squares Between Groups + Sum of Squares Within Groups.

Total Sum of Squares = Sum of Squares Between Groups + Sum of Squares Within Groups

Example- one way ANOVA

Example: 3 samples obtained from normal populations with equal variances. Test the hypothesis that sample means are equal

8	7	12
10	5	9
7	10	13
14	9	12
11	9	14

1. Null hypothesis –

No significant difference in the means of 3 samples

2. State Alpha i.e 0.05

3. Calculate degrees of Freedom

$$k-1 \quad \& \quad n-k = 2 \quad \& \quad 12$$

4. State decision rule

Table value of F at 5% level of significance for d.f 2 & 12 is 3.88

The calculated value of $F > 3.88$, H_0 will be rejected

5. Calculate test statistic

X1	X2	X3
8	7	12
10	5	9
7	10	13
14	9	12
11	9	14
Total 50 M1= 10	40 M2 = 8	60 M3 = 12

$$\text{Grand average} = \frac{10 + 8 + 12}{3} = 10$$

Variance BETWEEN samples (M1=10, M2=8, M3=12)

Sum of squares between samples (SSC) =

$$n_1 (\mathbf{M1} - \text{Grand avg})^2 + n_2 (\mathbf{M2} - \text{Grand avg})^2 + n_3 (\mathbf{M3} - \text{Grand avg})^2 \\ 5 (\mathbf{10} - 10)^2 + 5 (\mathbf{8} - 10)^2 + 5 (\mathbf{12} - 10)^2 = 40$$

Calculation of Mean sum of Squares between samples (MSC)

$$MSC = \frac{SSC}{k - 1} = \frac{40}{2} = 20$$

k= No of Samples, n= Total No of observations

Variance WITH IN samples (M1=10, M2=8, M3=12)

X1	$(X1 - M1)^2$	X2	$(X2 - M2)^2$	X3	$(X3 - M3)^2$
8	4	7	1	12	0
10	0	5	9	9	9
7	9	10	4	13	1
14	16	9	1	12	0
11	1	9	1	14	4
	30		16		14

Sum of squares within samples (SSE) = 30 + 16 + 14 = 60

Calculation of Mean Sum Of Squares within samples (MSE)

$$MSE = \frac{SSE}{n - k} = \frac{60}{12} = 5$$

Calculation of ratio F

$$F = \frac{\text{Variability between groups}}{\text{Variability within groups}}$$

$$F\text{- statistic} = \frac{MSC}{MSE} = 20/5 = 4$$

The Table value of F at 5% level of significance for d.f 2 & 12 is 3.88
The calculated value of F > table value
H₀ is rejected. Hence there is significant difference in sample means

Short cut method -



X1	(X1) ²	X2	(X2) ²	X3	(X3) ²
8	64	7	49	12	144
10	100	5	25	9	81
7	49	10	100	13	169
14	196	9	81	12	144
11	121	9	81	14	196
Total 50	530	40	336	60	734

Total sum of all observations = $50 + 40 + 60 = 150$

Correction factor = $T^2 / N = (150)^2 / 15 = 22500 / 15 = 1500$

Total sum of squares = $530 + 336 + 734 - 1500 = 100$

Sum of square b/w samples = $(50)^2 / 5 + (40)^2 / 5 + (60)^2 / 5 - 1500 = 40$

Sum of squares within samples = $100 - 40 = 60$

Example with SPSS

Example:

Do people with private health insurance visit their Physicians more frequently than people with no insurance or other types of insurance ?

N=86

- **Type of insurance** - 1.No insurance
2.Private insurance
3. TRICARE
- No. of visits to their Physicians(dependent variable)

Violations of Assumptions

Normality

Choose the non-parametric **Kruskal-Wallis H Test** which does not require the assumption of normality.

Homogeneity of variances

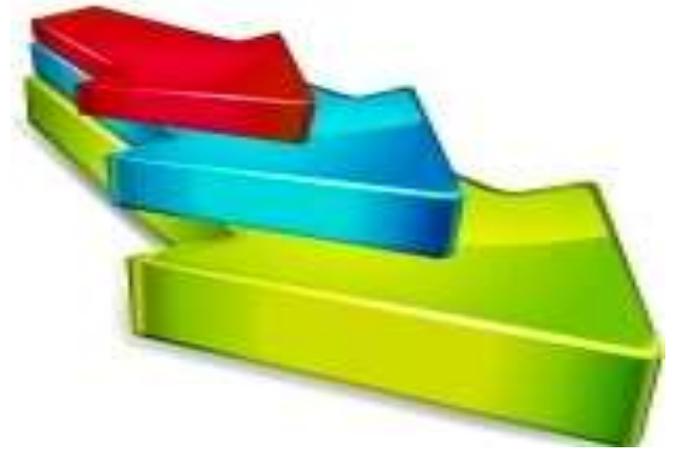
- ✓ Welch test or
- ✓ Brown and Forsythe test or Kruskal-Wallis H Test



Two Way ANOVA

Data required

- When 2 independent variables (Nominal/categorical) have an effect on one dependent variable (ordinal or ratio measurement scale)
- Compares relative influences on Dependent Variable
- Examine interactions between independent variables
- Just as we had Sums of Squares and Mean Squares in **One-way ANOVA**, we have the same in **Two-way ANOVA**.



Two way ANOVA

Include tests of three null hypotheses:

- 1) Means of observations grouped by one factor are same;
- 2) Means of observations grouped by the other factor are the same; and
- 3) There is no interaction between the two factors. The interaction test tells whether the effects of one factor depend on the other factor

Example-

we have test score of boys & girls in age group of 10 yr, 11yr & 12 yr. If we want to study the effect of gender & age on score.

Two independent factors- Gender, Age

Dependent factor - Test score

Ho - Gender will have no significant effect on student score

Ha -

Ho - Age will have no significant effect on student score

Ha -

Ho – Gender & age interaction will have no significant effect on student score

Ha -

Two-way ANOVA Table

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F-ratio	P-value
Factor A	$r - 1$	SS_A	MS_A	$F_A = MS_A / MS_E$	Tail area
Factor B	$c - 1$	SS_B	MS_B	$F_B = MS_B / MS_E$	Tail area
Interaction	$(r - 1)(c - 1)$	SS_{AB}	MS_{AB}	$F_{AB} = MS_{AB} / MS_E$	Tail area
Error (within)	$rc(n - 1)$	SS_E	MS_E		
Total	$rcn - 1$	SS_T			

Example with SPSS

Example:

Do people with private health insurance visit their Physicians more frequently than people with no insurance or other types of insurance ?

N=86

- **Type of insurance** - 1.No insurance
2.Private insurance
3. TRICARE
- No. of visits to their Physicians(dependent variable)

Gender

0-M

1-F



MANOVA

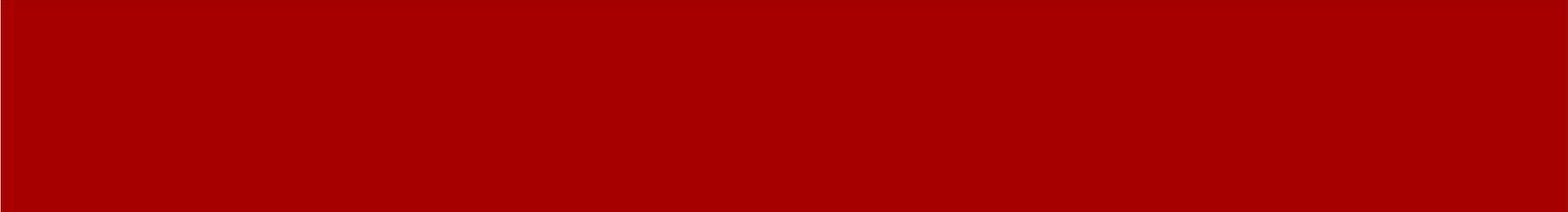
Multivariate ANalysis Of VAriance

Data Required

- MANOVA is used to test the significance of the effects of one or more IVs on **two or more DVs**.
- It can be viewed as an extension of ANOVA with the key difference that we are dealing with **many dependent variables** (not a single DV as in the case of ANOVA)

- **Dependent Variables (at least 2)**
 - Interval /or ratio measurement scale
 - May be correlated
 - Multivariate normality
 - Homogeneity of variance
- **Independent Variables (at least 1)**
 - Nominal measurement scale
 - Each independent variable should be independent of each other

- Combination of dependent variables is called **“joint distribution”**
- MANOVA gives answer to question
“ Is joint distribution of 2 or more DVs significantly related to one or more factors?”

- 
- The result of a MANOVA simply tells us that a difference exists (or not) across groups.
 - It does not tell us which treatment(s) differ or what is contributing to the differences.
 - For such information, we need to run ANOVAs with post hoc tests.

Various tests used-

✓ **Wilk's Lambda**

- Widely used; good balance between power and assumptions

✓ **Pillai's Trace**

- Useful when sample sizes are small, cell sizes are unequal, or covariances are not homogeneous

✓ **Hotelling's (Lawley-Hotelling) Trace**

- Useful when examining differences between two groups

Example with SPSS

Example:

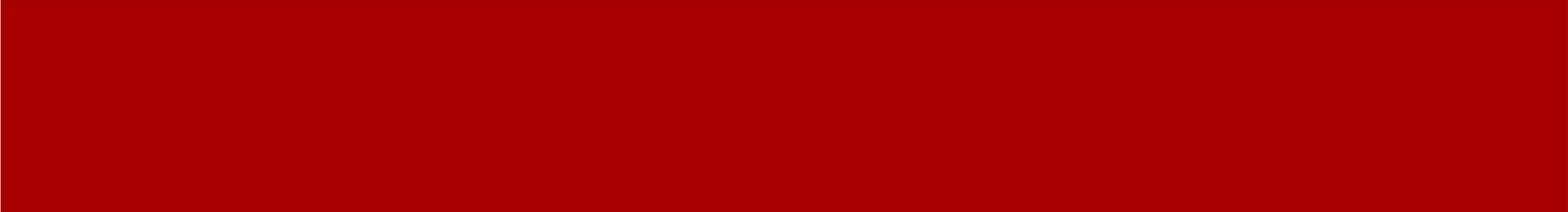
Do people with private health insurance visit their Physicians more frequently than people with no insurance or other types of insurance ?

N=50

Gender(0-M,1-F)

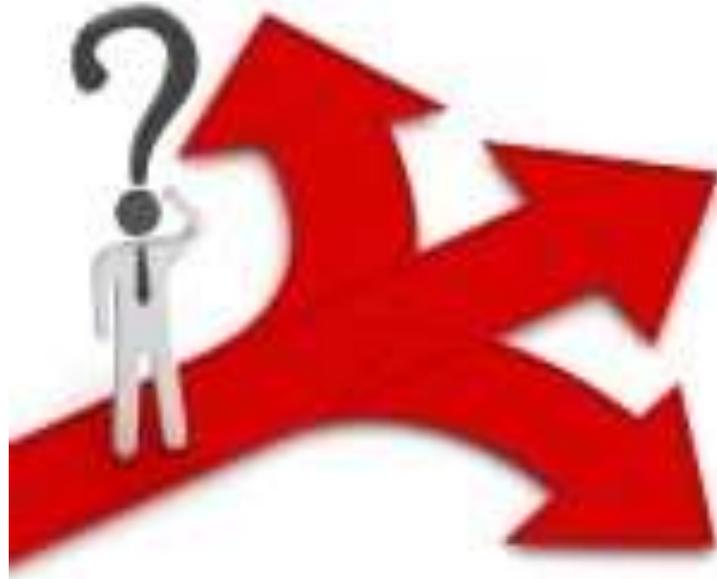
- **Type of insurance** - 1.No insurance
2.Private insurance
3. TRICARE
- No. of visits to their Physicians(dependent variable)

Satisfaction with facility provided



Research question

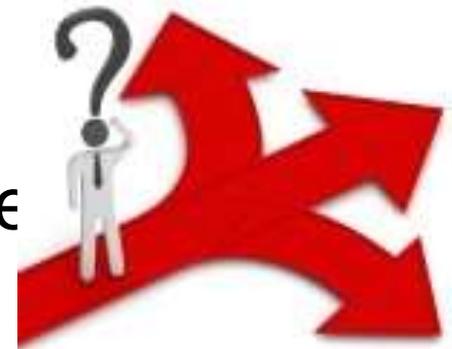
1. Do men & women differ significantly from each other in their satisfaction with health care provider & no. of visits they made to a doctor
2. Do 3 insurance groups differ significantly from each other in their satisfaction with health care provider & no. of visits they made to a doctor
3. Is there any interaction b/w gender & insurance status in relation to satisfaction with health care provider & no. of visits they made to a doctor



ANOVA with repeated measures

ANOVA with Repeated Measures

- Determines whether means of 3 or more measures from same person or matched controls are similar or different.
- Measures DV for various levels of one or more IVs
- Used when we repeatedly measure the same subjects multiple times



Assumptions

- Dependent variable is **interval /ratio (continuous)**
- Dependent variable is **approximately normally distributed.**
- One independent variable where participants are tested on the same dependent variable at least 2 times.
- **Sphericity-** condition where variances of the differences between all combinations of related groups (levels) are equal.

Sphericity violation

- Sphericity can be like homogeneity of variances in a between-subjects ANOVA.
- The violation of sphericity is serious for the Repeated Measures ANOVA, with violation causing the test to have an increase in the Type I error rate).
- **Mauchly's Test of Sphericity** tests the assumption of sphericity.

Sphericity violation

- The corrections employed to combat violation of the assumption of sphericity are:
 - ✓ **Lower-bound estimate,**
 - ✓ **Greenhouse-Geisser correction** and
 - ✓ **Huynh-Feldt correction.**
- The corrections are applied to the degrees of freedom (df) such that a valid critical F-value can be obtained.

Steps ANOVA

1. Define null & alternative hypotheses

2. State Alpha

3. Calculate degrees of Freedom

4. State decision rule

5. Calculate test statistic

- Calculate variance between samples
- Calculate variance within the samples
- Calculate ratio F
- If F is significant, perform *post hoc* test

6. State Results & conclusion

Calculate Degrees of Freedom for

- D.f between samples = $K-1$
- D.f within samples = $n- k$
- D.f subjects= $r -1$
- D.f error= d.f within- d.f subjects
- D.f total = $n-1$

State decision rule

If calculated value of $F >$ table value of F , reject H_0

Calculate test statistic ($f = MS_{bw} / MS_{error}$)

	SS	DF	MS	F
Between				
Within				
-subjects				
- error				
Total				

State Results & conclusion

Example with SPSS

Example-

Researcher wants to observe the effect of medication on free T 3 levels before, after 6 week, after 12 week. Level of free T 3 obtained through blood samples. Are there any differences between 3 conditions using alpha 0.05?

- ✓ **Independent Variable- time 1, time 2, time 3**
- ✓ **Dependent Variable- Free T3 level**

Other related tests-

ANCOVA (Analysis of Covariance)

Additional assumptions-

- Covariate should be continuous variable
- Covariate & dependent variable must show a linear relationship & must be similar in each group

MANCOVA (Multivariate analysis of covariance)

One or more continuous covariates present

References

- Methods in Biostatistics *by* BK Mahajan
- Statistical Methods *by* SP Gupta
- Basic & Clinical Biostatistics *by* Dawson and Beth
- Munro's statistical methods for health care research

Thank you!
Jimmy

